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(54) Thioether adsorbent intended for the separation of proteins and the like.

(57) An adsorbent, characterized by a carrier with covalently bound ligands consisting of a solid phase which is completely or partially penetrated by or surface coated with a hydrophilic, molecular, polymeric netting.

The bound ligands consist of atom or atomic group Y and a group X located terminally from Y in the ligand, wherein Y and X are separated from each other by two consecutively bonded carbon atoms and wherein X is SO_2, SO, S or Se, and wherein Y is S, N, NR or NR_2, where R is hydrogen or alkyl.

The solid phase is formed of particles preferably of smaller diameter than 1 mm, and the polymeric lattice is formed of a polyhydroxy polymer such as a polygalactane, e.g. agar or agarose, or a polyamide such as a cross-linked polyacryl amide or a derivative thereof.

The ligand has the structure R_1-X-CH_2-CH_2-S- where R_1 is an arbitrary aliphatic, aromatic or heterocyclic group having for example the structure $-(CH_2)_m-(CHQ)_n-H$ wherein Q is H, OH or SH and wherein n = 1, 2 or a higher number and m = 0, 1 or a higher number.

The adsorbent is prepared by first introducing thiol groups into the polymeric netting for subsequent alkylization with vinyl-β-haloethyl sulfone, -sulfoxide or sulfide.

The adsorbent can be used, with an excellent result, for the fractionation of biopolymers such as proteins, for example.

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THIOETHER ADSORBENT INTENDED FOR THE SEPARATION OF PROTEINS AND THE LIKE

Different kinds of adsorbents are used within the fields of biochemistry and biotechnology for the isolation of macro- as well as "micromolecules" and for the immobilization of e.g. enzymes for technical applications, as well as antigens and antibodies for the diagnosis of diseases. The present invention belongs to this category of adsorbents but distinguishes from conventional types of adsorption agents as to certain characteristic properties.

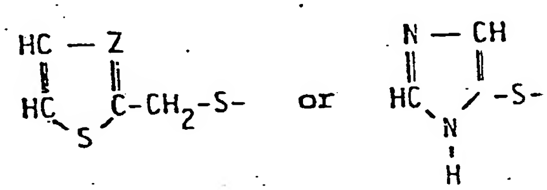
The invention relates to a finely divided adsorbent consisting of particles having in a particularly useful form a spherical configuration with a diameter in the magnitude of 1 - 1000 µm. The particles are distinguished by their structure of a polymeric netting penetrating the entire particles or acting as a surface coating, and containing substituents having the structure

$$-X-\overset{|}{\underset{|}{C}}-CH_2-Y- \quad \text{or} \quad -X-\overset{|}{C}=CH-Y- \qquad \text{(I)}$$

wherein X can be $>SO_2$, $>SO$, $>S$ or $>Se$ and Y is $>S$ or NR, for example

$$R-X-CH_2-CH_2-S- \qquad \text{(II)}$$

and



(III)

wherein Z is N or CH, and R can be an arbitrary substituent.

A distinguishing feature of the invention is its adsorption properties, the nature of which can be of a kind similar to that of so-called hydrophobic adsorbents. For example, the adsorption of proteins is increased in the presence of high concentrations of aqueous structure

organized salts such as e.g. alkali chlorides, alkali sulfates and magnesium sulfates. Apparently however, adsorbents according to the invention preferably adsorb other proteins than is the case with hydrophobic adsorbents based on the presence of alkyl groups containing e.g. 8 - 18 carbon atoms.

An unexpected and surprising discovery is that R can be hydrophilic, e.g. $-CH_2-CH_2-OH$, or even $-CH_2-CHOH-CH_2OH$, which indicates that a characteristic and hitherto unknown adsorption effect is connected with the structure

$$-X-\overset{|}{\underset{|}{C}}-CH_2-S-,$$

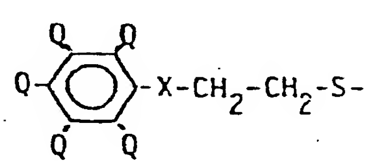
this effect being distinguished from the hydrophobic adsorption.

This interaction may be called thiophilic ("attracted to sulfur"), said adsorption thus being a thiophilic adsorption.

R may be an unsubstituted alkyl group $CH_3(CH_2)_n-$. If n is a large integer there are obtained elements of hydrophobic as well as thiophilic adsorption where the hydrophobic adsorption tends to become predominant along with the increasing n. For n=o, i.e. R = methyl, there is practically obtained nothing but a thiophilic adsorption.

Consequently, the total adsorption effect can be modified in response to the chemical character of R. If a carboxyl group is introduced, the thiophilic adsorption is damped and the charge-dependent adsorption tends to take over.

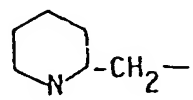
The R group can be aromatic, e.g. phenyl (Example 6). Strangely enough, the adsorption of proteins to a product according to the invention, having the structure



(IV)

and with x containing S or SE, is most markedly distinguished from e.g. analogous adsorbents where X is -O- or -NH-.

The R group can be a heterocyclic group directly or indirectly bound to X, for example



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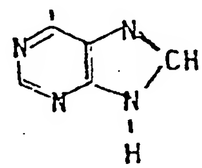
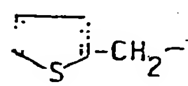


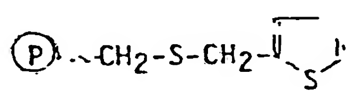
(VI)

The heterocyclic R groups may also comprise thenyl;



(VII)

although thenyl may be directly attached to a thiol group in the matrix:

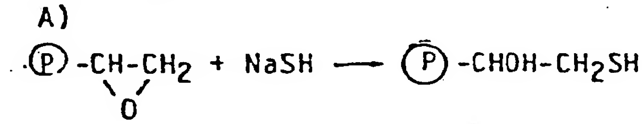


(VIII)

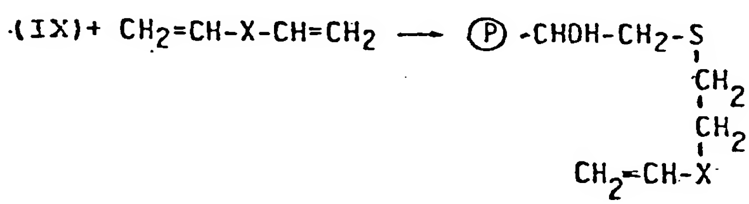
The adsorption is modified in various manners by the heterocyclic groups, but the thiophilic adsorption, manifested by the dependency on salt, will always remain.

Adsorbents according to the invention can be prepared from a thiol- or epoxide-containing carrier:

A)



(IX)



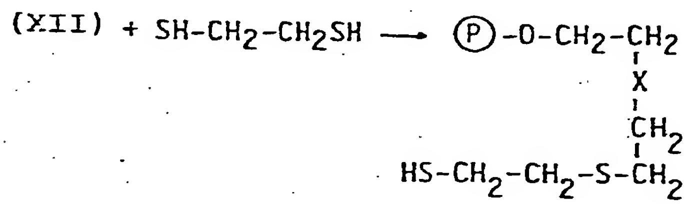
(X)

$$(X) + HS\text{-}CH_2\text{-}CH_2OH \longrightarrow$$

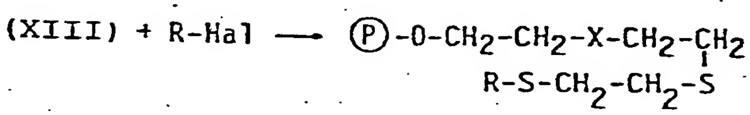
$$Ⓟ\text{-}CHOH\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}X\text{-}CH_2\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH_2OH \quad (XI)$$

B)

$$Ⓟ\text{-}OH + CH_2\text{=}CH\text{-}X\text{-}CH_2 \longrightarrow Ⓟ\text{-}O\text{-}CH_2\text{-}CH_2\text{-}X\text{-}CH\text{=}CH_2 \quad (XII)$$

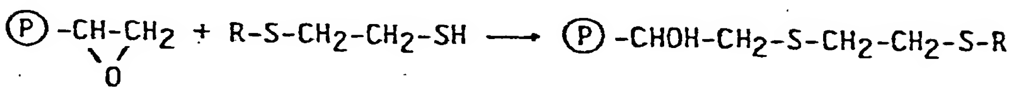


(XIII)



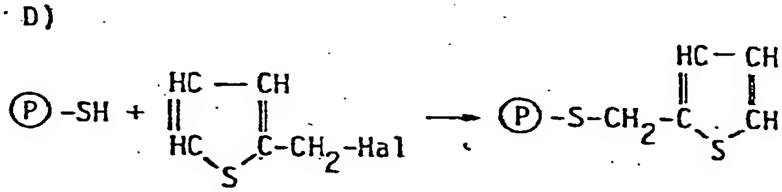
(XIV)

C)



(XV)

D)



(XVI)

It is also possible in principle to prepare the inventive adsorbents directly from a hydroxyl- or amino group-containing carrier, for example

$$Ⓟ\text{-}OH + CH_2\text{=}CH\text{-}X\text{-}CH_2\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}X\text{-}R \longrightarrow$$

$$Ⓟ\text{-}O\text{-}CH_2\text{-}CH_2\text{-}X\text{-}CH_2\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}X\text{-}R \quad (XVII)$$

It appears from the aforegoing that one embodiment of the invention can consist of particles constituting a netting for proteins which is permeable all the way to the center. In accordance with another alternative, only the surface layer of the particle is permeable for proteins, and in such a case the particle nucleus does not have to contain the group which is characteristic for adsorption. A third alternative can consist of a more or less heavy layer of netting surrounding an impermeable nucleus. The nucleus can be arbitrarily formed and constitute e.g. a fiber, the interior surface of a hose, a beaker or some other vessel, etc.

For the permeation of macromolecules and for the appropriate appearance of the distinguishing adsorption characteristics, the polymeric netting must have a specific property; it must be hydrophilic and permeable for the macromolecules, it must be resistent within a pH range where proteins can be adsorbed without being damaged, preferably within the range of pH 4-8 but suitably within a wider range. The chemical properties of the polymeric netting must also be well adapted to allow introduction of the group characteristic for the adsorption. With regard to the polymeric netting, the invention is restricted by these limitations to hydrophilic polymers of the following types: polyalcohols such as e.g. polyvinyl alcohols, polyhydroxy methanes, polysaccharides, cross-linked hexitholes and polyamides such as cross-linked polyacryl amide. Also cross-linked polyamines and polyacides can be mentioned, although it should then be observed that the adsorption will be more complicated by the presence of the ionogenic groups. The gel netting can furthermore consist of inorganic gels to which have been coupled organic hydroxyl-containing substituents, for example substituted silicic acid gels, or groups such as

$$
\begin{array}{l}
\quad\;\; CH_3 \\
\quad\;\;\, | \\
O-Si-CH_2-CH_2-CH_2-NH- \\
\quad\;\;\, | \\
\quad\;\; CH_3
\end{array}
$$

A particularly useful adsorbent according to the invention consists of cross-linked agar.

Example 1

Particulate 6% agarose gel was treated in a reaction vessel with 5% butanediol-bis-glycidyl ether in 0.6 M NaOH for 2 hours at 40°C. The gel was washed and then converted to thiol derivative by treatment with NaSH in 1 M Na_2CO_3 in the presence of 0.1% $NaBH_4$. The gel, "thiol agarose", was washed with distilled water followed by 0.1 M $NaHCO_3$, and was transferred to a reaction vessel for treatment during 1 hour with 1% divinyl sulfone. To the thiol agarose, treated with divinyl sulfone, was added excess mercapto ethanol and the mixture was allowed to react for 2 hours in 0.1 M $NaHCO_3$.

The gel was tested with regard to its ability of absorbing proteins. A gel bed of 5 ml was prepared and washed with 0.1 M Tris-HCl, 0.5 M K_2SO_4, at pH 7.6. Human serum dialyzed against this buffer solution to an amount of 50 absorbency properties (280 nm) was introduced into the bed, whereafter the gel was washed with 50 ml of buffer salt solution. Adsorbed protein was thereafter desorbed by first washing with 0.1 M tris-HCl, pH 7.6 (without K_2SO_4) and then with 40% ehtylene glycol, with 30% isopropanol, and finally with 95% ethanol. 45% protein was adsorbed to the gel of which 25% was eluated with the sulfate-free buffer and the remaining part with other eluents.

It was shown by electrophoretic analysis that all serum albumine passed the gel without adsorption, whereas immunoglobulin was fixed to the gel and was eluated by the salt-free buffer. α_2-macroglobuline as well as lipoproteins were also completely adsorbed.

Example 2

Example 1 was repeated but with the difference that cellulose powder was used instead of agarose particles.

Example 3

A trial was carried out with epichlorohydrin-treated polyacryl amide (commercial Eupergite). The oxirane gel

was converted to SH-gel with NaSH, which was then treated with divinyl sulfone and thereafter with mercapto ethanol. The polyacrylamide derivative had a somewhat lower capacity than had the agarose gel according to Example 1, but the selectivity was principally the same.

Example 4

Thiol agarose was activated with divinyl sulfone followed by the attachment of 2.3-dihydroxy-propane-1-thiol as described in Example 1. In an adsorption test with human serum, 43% of the proteins having the same selectivity pattern as in Examples 1-3 was adsorbed but with the difference that the desorption with ethylene glycol was more efficient (10% compared to 6% in Example 1).

Example 5

The same procedure was employed as in Example 4 but with the use of 2.3-dimercapto-1-propanol. The adsorption capacity was lower (32%).

Example 6

Epichlorohydrin-activated agarose was converted into thiol gel and was treated with phenylvinyl sulfone in 0.1 M $NaHCO_3$. 20% serum protein was adsorbed on the gel, mainly by thiophilic adsorption as it was obvious that all albumine passed the bed without adhering.

Example 7

Thiolgel according to previous examples was converted as described in Example 6 but with the difference that the phenylvinyl sulfone was exchanged for phenyl vinyl sulfoxide. The capacity was somewhat higher as compared with corresponding sulfone derviative (22%), although the selectivity was the same.

Example 8

Thiolagarose was divinylsulfone-activated as in Example 1 and was then converted with thioglycolic acid in 0.1 M $NaHCO_3$. The product was found to adsorb less than 5% protein. After esterification, the capacity was increased to 37% although the yield at desorption was low; total yield about 75%.

Example 9

Divinylsulfone-activated thiolagarose was converted with selenoglycolic acid analogously with Example 8. The adsorption capacity was low both prior to and after esterification. Reduction with $NaBH_4$ in LiCl solution resulted in an extremely high improvement of the adsorption capacity (48% increase). It proved very difficult however to desorb adhered protein (total yield 63%).

Example 10

Divinylsulfone-activated thiolagarose was linked with 2.2'-diehtylamino ethane thiol in 0.1 M $NaHCO_3$. The product obtained was found to have an extremely low adsorption capacity (13%). Liptoproteins in principle were fixed to the gel.

Example 11

Agarose was activated with thosyl chloride in alkali and was then treated with 1.2-ethane thiol followed by methyl iodide. The product was found to have good capacity (43%). 36% of the adsorbed material was desorbed by the sulfate-free buffer. The total yield was quantitative.

CLAIMS

1. Adsorbent intended for the separation and immobilization of proteins, consisting of a carrier with covalently bound ligands, wherein the carrier constitutes a solid phase which is completely or partially penetrated by or surface-coated with a hydrophilic, molecular polymeric netting, characterized in that the bound ligands consist of the groups

$$R - X - CH_2 - CH_2 - Y -$$

wherein R is $H - (CHQ)_n - (CH_2)_m -$

where $Q = H, OH, SH$;

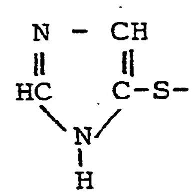
$n = 0, 1, 2$; and

$m = 0, 1$;

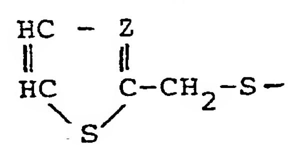
or an alkyl, hydroxy alkyl, mercapto alkyl or a phenyl group containing a nitro group, or hydroxyl or amino group unsubstituted or substituted with alkyl, hydroxy alkyl or acyl in ortho- or para-position to a thioether atom or a heterocyclic, nitrogen-containing ring;

$X = SO_2$, SO, S or Se; or

RX is formed of a heterocyclic ring



or



where Z = N or CH; and

Y = S, N, NR_1 wherein R_1 is hydrogen or alkyl.

2. Adsorbent as claimed in Claim 1, characterized in that the solid phase is formed of particles having a diameter which is preferably smaller than 1 mm.

3. Adsorbent as claimed in Claims 1 and 2, characterized in that the polymeric netting is formed of a polyhydroxy polymer.

4. Adsorbent as claimed in any one of Claims 1-3,

characterized in that the polyhydroxy polymer is a polygalactane, for example agar or agarose.

5. Adsorbent as claimed in Claim 1 or 2, characterized in that the polymeric netting is formed of a polyamide, for example cross-linked polyacryl amide or a derivative thereof.

6. Method of preparing an absorbent as claimed in any one of Claims 1-5, characterized in that thiol groups are first introduced into the polymeric netting for subsequent alkylization with vinyl-β-haloethyl sulfone, -sulfoxide or -sulfide.

7. Utilization of the adsorbent as claimed in Claim 1 for biopolymeric fractionation.

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EUROPEAN SEARCH REPORT

EP 85850148.9

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
A	GB - A - 2 006 640 (EASTMAN KODAK) * Examples; claims *	1,3,4, 7	B 01 J 20/32 B 01 D 15/00 G 01 N 30/48
A	GB - A - 2 092 470 (TANABLE SEI-YAKU) * Claims *	1,3,4, 7	
A	US - A - 3 917 527 (SHALTIEL) * Claims *	1,3,4, 7	
A	GB - A - 1 544 867 (INSTITUT MERIEUX) * Examples; claims *	1,3,4, 7	

TECHNICAL FIELDS SEARCHED (Int. Cl 4)

B 01 J
B 01 D
G 01 N
C 12 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	06-11-1986	TENGLER

CATEGORY OF CITED DOCUMENTS

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